TEMPERATURE DEPENDENCY OF THE RATE OF ELECTRON TRANSPORT AS A MONITOR OF PROTEIN MOTION

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ABSTRACT The temperature dependency of the rate of biological electron transport is interpreted as evolving from a contraction of the electron transport components. A theoretical expression for this temperature dependency is derived in terms of the coefficient of linear expansion (α) of the protein components. Using this expression α is calculated for several electron transport systems and shown to be similar to α-values of synthetic polymers. A discontinuity in α is shown to be present in all biological electron transport reactions at ca. 150 K. This discontinuity is interpreted as a change in the intramolecular bonding of the electron transport protein units.

Since the first experiments (1, 2) in biological electron transport at cryogenic temperatures, much discussion has arisen concerning the mechanism for such transport at these low temperatures. In an attempt to better understand the mechanism of electron transport, DeVault and Chance (3, 4) measured the temperature dependency of the rate of transfer of an electron from reduced cytochrome *c* to the oxidized primary donor unit in whole cells of the photosynthetic bacterium *Chromatium* D. Their results show that the rate of this transfer decreases with decreasing temperature following the normal temperature dependency of a reaction dependent on an activation energy. Below ca. 150 K, however, they observed that the rate of transfer remained constant. The temperature-independent region of this rate was interpreted by them to represent a quantum mechanical tunneling of the electron through the potential barrier of the lipid-protein environment. Since electron tunneling in this temperature region depends mainly on the height and width of the potential barrier separating the electron donor and acceptor species and not on an activation energy, such a process is presumably temperature independent.

Recently, a large number of papers have presented data on the temperature dependency of various electron transport reactions, albeit all of these reactions are associated with photosynthetic systems. The purpose of this paper is to summarize all of this data in order to show how the temperature dependency of the various rates of electron transport reactions can be interpreted in terms of a general theory of the temperature dependency of protein motions.

The temperature effects on electron transport can most easily be visualized in terms

of a rate plot. A typical rate plot is shown in Fig. 1 where the half-life ($t_{1/2}$) of various biological electron transport reactions are plotted on a logarithm scale against the absolute temperature. Decay half-lives are inversely proportional to the rate constants for the reactions described in this paper since each is first-order in kinetics. In discussing the temperature dependency of an electron transport reaction, it is most convenient to section the rate plot of that reaction into three main temperature regions: (*1*) a high temperature region (typically from ca. 150 K to room temperature and called region 1); (*2*) a low temperature region (below ca. 150 K and called region 2); and (*3*) and the temperature of transition between these two regions called the inflection temperature (T_{inf}). The value of T_{inf} can be easily obtained from the rate plot by first drawing the best straight lines through the data in regions 1 and 2. The temperature at which these lines intersect defines T_{inf} (see Fig. 1).

As mentioned above, region 2 was first investigated by DeVault and Chance (3, 4) whose data is represented by the solid squares in Fig. 1. The rate of electron transport in this region has little, and in many cases no, temperature dependency. These workers suggested that the persistence of this reaction to occur at such low temperatures can best be rationalized in terms of the electron tunneling through the potential barrier of the lipid-protein environment from the donor (reduced cyt *c*) to the acceptor (the oxidized bacteriochlorophyll donor unit). This explanation is still widely accepted today (6).

On the other hand, the temperature dependency observed in region 1 is much less well understood. DeVault and Chance interpreted their result in this region in terms of a system requiring an activation energy but also suggested a possible second mechanism. They pointed out that the rate of tunneling through a barrier may be a function of a third parameter in addition to the magnitude of the barrier height and width. The third parameter involves the matching of the electron donor and acceptor energy levels on either side of the barrier. The better the match, the faster the transfer. This view of biological electron transport is similar to the theory of solution oxidation-reduction reactions formalized by Marcus (7, 8) who stated that the transport of an electron is influenced by the amount of donor and acceptor overlap. Chernavskii and coworkers (9, 10) have similarly discussed the possible involvement of phonons and normal vibrations in the regulation of the temperature dependency in region 1. Using a theoretical argument, they suggest that the temperature effects arising from a matching of the vibronic levels on either side of the barrier will mirror those effects typically associated with a process requiring an activation energy and thus can also explain the temperature dependency in region 1. Hopfield (11) has recently expanded this argument to include a factor proportional to the frequency of the electron approaching the barrier. Unfortunately, fitting his rate equation to experimental data predicts a barrier width of 8–10 Å which is smaller than the dimensions of a typical electron transport protein (e.g., cytochrome *c* is 25 × 25 × 35 Å).

The arguments used by these authors to explain the temperature dependency in region 1 suffers from two serious flaws. First, the data showing systems with an apparent negative activation energy (i.e., a rate increase with decreasing temperature as

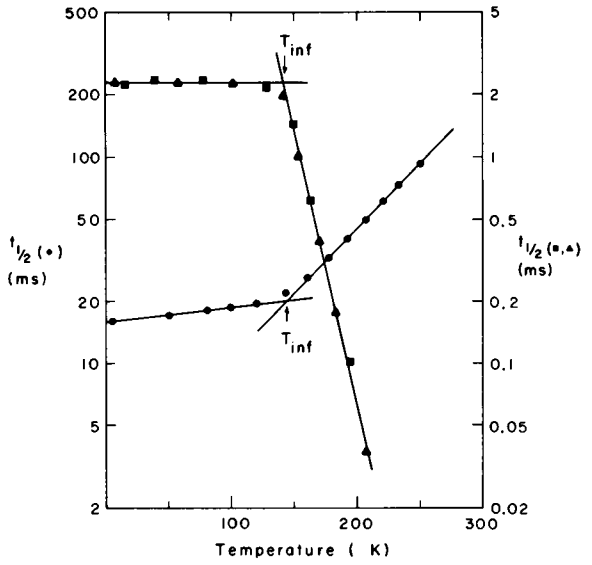

FIGURE 1

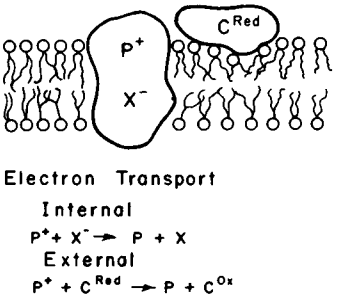

FIGURE 2

FIGURE 1 Rate plot of electron transport reactions. Decay half-times ($t_{1/2}$) are plotted on a logarithm scale vs. the absolute temperature. Data is plotted for the back transport of an electron from the reduced primary acceptor to the oxidized primary donor in *R. rubrum*. (●, ref. 5) and corresponds to the left-hand scale as well as for the electron transport from reduced cytochrome *c* to the oxidized primary donor in *Chromatium* D (■, ref. 3 and ▲, B. J. Hales, unpublished results) which corresponds to right-hand scale.

FIGURE 2 Schematic of a biological membrane showing electron transport which can be internal (one protein) or external (two different proteins). *P*, primary photosynthetic electron donor; *X*, primary electron acceptor; *C*, cytochrome *c* in oxidized (ox) and reduced (red) states.

depicted by the solid circles in Fig. 1) were not considered. Their theories obviously cannot explain such a phenomenon. Second, one would expect that the energy separation between the vibronic levels in biological systems of such large molecular weight to be so small as to be insignificant in regulating the rate of electron tunneling except at temperatures much lower than 150 K.

Recently, a third explanation for the temperature dependency in region 1 has been suggested (6, 12) by several research groups. If one considers electron transport to be a tunneling process at all temperatures below room temperature instead of just below 150 K then the temperature dependency of the transfer half-life can be expressed in terms of temperature related changes in the lipid-protein barrier width and height. More exactly, the half-life of a tunneling process (3, 6) through a square potential barrier can be written as:

$$t_{1/2} = [\ln(2)\,\pi\hbar V^2/8E^2(V\text{-}E)]e^{[2W_T[2m(V\text{-}E)]^{1/2}/\hbar]}, \quad (1)$$

or

$$\ln(t_{1/2}) = (2W_T[2m(V\text{-}E)]^{1/2}/\hbar) + \ln[\ln(2)\pi\hbar V^2/8E^2(V\text{-}E)], \quad (2)$$

where $t_{1/2}$ is transport half-life; E, energy of electron; V, barrier height; W_T barrier width at absolute temperature T; and m, mass of electron.

Before this equation can be used, a number of approximations and assumptions must be made: (*a*) since all of the reactions investigated are in biological systems, V and E should be the same order of magnitude for all of these systems; (*b*) only the magnitude V, E, and W_T will be effected by changes in temperature; (*c*) as will be suggested below $\frac{1}{2}V \simeq E \simeq \frac{1}{2}eV$ so that V and E are virtually temperature invariant below 300 K; (*d*) a fixed temperature-independent frequency factor is assumed for Eqs. 1 and 2. Using these assumptions the temperature variation of the rate of electron transport can be explained in terms of changes in the magnitude of the barrier width, W_T, for a given electron transport reaction, or Eq. 2 can be simplified to

$$\ln(t_{1/2}) = A W_T + B, \quad (3)$$

where

$$A = 2[2m(V\text{-}E)]^{1/2}/\hbar, \quad (4)$$

$$B = \ln[\ln(2)\pi\hbar V^2/8E^2(V\text{-}E)] \quad (5)$$

are characteristic constants for a given reaction and are assumed to have little temperature dependency. The variation in W_T with temperature can be conveniently expressed in terms of the coefficient of linear thermal expansion, α, which is defined by

$$W_T = W_0 + W_0\alpha T, \quad (6)$$

where W_0 is the hypothetical barrier width at 0 K.

Therefore,

$$\ln(t_{1/2}) = \beta T + \gamma, \quad (7)$$

where

$$\beta = A W_0\alpha, \quad (8)$$

$$\gamma = A W_0 + B. \quad (9)$$

Since A and W_0 are positive, the sign of β depends on the sign of α.

Fig. 1 shows the linear dependency of $\ln(t_{1/2})$ on temperature in region 1 for two sample electron transport reactions as predicted by Eq. 7. Unlike the model employing an activation energy in which $\ln(t_{1/2})$ would be inversely proportional to temperature, Eq. 7 can explain both positive and negative slopes, β, for the rate plots in region 1. Those systems in which cooling causes the electron transport components to move closer together will have a positive slope β in region 1 while those systems those electron transport components separate upon cooling will have negative slopes. If an electron transport reaction involves redox groups in a single protein (see Fig. 2), it would be expected that cooling would cause contraction of that protein, which would

move the redox groups closer together; such a system would have a positive β. On the other hand, redox groups in different proteins (Fig. 2) have the ability to (although will not always) move further apart upon cooling and yield a system with a negative β. For such a phenomenon to occur, the coefficient of thermal expansion of the medium separating the proteins must be less than that of the proteins. In such a system, if the far side of each protein is bound to the medium, cooling will cause a separation of the proteins.

If the above argument is correct, then the magnitude of β obtained from experimental data could be used in the calculation of the magnitude of α. From the experimental data shown in Fig. 1, β can be calculated to equal 0.0145 K^{-1} for the back electron transfer from the primary acceptor to the donor in *Rhodospirillum rubrum* (transport 1) and -0.0614 K^{-1} for the transfer between reduced cytochrome *c* and the oxidized donor unit in, *Chromatium* D. (transport 2). As stated above, the magnitude of both A and B are assumed to be independent of the electron transport system being investigated. If we make the further assumption (3, 6) that $\frac{1}{2}V = E \simeq \frac{1}{2}eV$, then A and B can be calculated using Eqs. 4 and 5 to be

$$A = 1.0 \times 10^{8}\,\mathrm{cm}^{-1},$$

$$B = 33.7.$$

The magnitude of W_0 can be determined by noting that 0 K Eq. 7 becomes

$$\ln(t_{1/2})_{T=0} = A W_0 + B,$$

or, upon rearrangement

$$W_0 = [\ln(t_{1/2})_{T=0} - B]/A. \tag{10}$$

Upon substitution of the calculated values of A and B, Eq. 10 becomes

$$W_0 = \ln(t_{1/2})_{T=0} + 33.7(\text{in Å}), \tag{11}$$

where $t_{1/2}$ is in the units of seconds. Values for $\ln(t_{1/2})_{T=0}$ can be obtained by extrapolating the data in region 1 to 0 K. The data in Fig. 1 yields $W_0 = 28$ Å for transport 1 and $W_0 = 36$ Å for transport 2.

Finally, using these values of A, β, and W_0, α can be calculated using Eq. 8 to be 5.2×10^{-4} K^{-1} for transport 1 and -17.1×10^{-4} K^{-1} for transport 2. Table I compares these α-values with those of some typical solid substances and shows that the biological α-values are of the same order of magnitude of those determined for several synthetic polymers.

Values of $t_{1/2}$, A, and B can now be used in Eq. 3 to determine the barrier width at room temperature (300 K); for transport 1, $W_{300} = 31$ Å and for transport 2, $W_{300} = 20$ Å. The magnitude of these numbers are in agreement with the observed greater efficiency of reduction of the oxidized donor unit in photosynthetic bacteria by reduced cytochrome *c* (transport 2) than by back electron transport from the reduced primary acceptor (transport 1).

TABLE I

COEFFICIENT OF LINEAR EXPANSION (α) OF VARIOUS SUBSTANCES

System	$\alpha \times 10^4$	Ref.
	K^{-1}	
Transport 1 (region 1)	5.2	This paper
Transport 1 (region 2)	0.42	This paper
Transport 2 (region 1)	−17.1	This paper
Polyethylene	2.3*	13
Cellulose	4.0*	13
Rubber (polyisoprene)	6.7*	13
Ice (253 K)	0.51	14
Aluminum	0.25	14

*Values represent one-third coefficient of volume thermal expansion.

Table II lists the sign of the β-term for various electron transport reactions. These reactions have been grouped according to their interpretation as being associated either with the back electron transport of the primary photochemical reaction of photosynthesis in bacterial or green plant and algae systems or with other secondary electron transport reactions. It is interesting to note that all the back reactions of the primary photochemical reaction in bacterial systems have positive β-terms while all the secondary reactions in both bacterial and green plant and algae systems have negative β-terms. Since the single protein containing the units involved in the primary photochemical reaction in photosynthetic bacteria has been isolated (22–24) and since the secondary reactions listed in Table II are known to involve different protein units, the sign of the β-term for both of these general systems is in agreement with the above theory. The negative β-term for the primary reaction in green plant and algae systems is not as easily accepted. According to the above theory, the negative β-term for this class of reactions means either that the reactions investigated are not the primary photochemical reactions as the various research groups have assumed or that the units involved in the primary photochemical reaction (both photosystem I and photosystem II) in green plant and algae systems are not housed in a single protein unit as has been shown in bacterial systems. This latter interpretation may help to explain the difficulty that has been encountered by various research groups in attempting to isolate a single photochemically active reaction center unit as has been done in bacterial systems.

Finally, the temperature separating regions 1 and 2 needs to be explained in terms of the above model. The inflection temperatures (T_{inf}) shown in Fig. 1 and listed in Table II is obviously a discontinuity in α. The coefficient of thermal expansion, α, will be discontinuous if the molecular binding of the medium suddenly changes at a given temperature. Although this explanation follows directly from the above discussion, it does not specify whether the discontinuity originates as a change in the intramolecular binding (or bonding) of the proteins, lipids, or water molecules within the medium or the intermolecular binding within several of these groups. Fortunately, this question

TABLE II

TEMPERATURE DEPENDENT PARAMETERS OF BIOLOGICAL ELECTRON TRANSPORT SYSTEMS

System	State	Sign (β)	T_{inf}	Average $t_{1/2}$ in region 2	Ref.
			K	*ms*	
Primary units, bacterial systems					
R. spheroides (UV-33)	Cph (dry film)	+	150		15
R. rubrum	Cph (ethyleneglycol-water, 4:6)	+	165	16–23	16
R. spheroides (R-26)	RC (glycerol)	+	190	20	17
R. spheroides (R-26)	RC (dry film)	+	165	20	17
R. rubrum	Cph	+	150	16–25	5
R. rubrum	AUT-e	+	150	16–25	5
R. spheroides (R-26)	Cph	+	150	30	12
R. spheroides (R-26)	RC	+	150	30	12
Primary units, green plants and algae					
Spinach	Cpl (system I)	–	150	350 (biphasic)	13
C. vulgaris	WC (system I)	–	150	350 (biphasic)	13
P. aerugineum	WC (system I)	–	150	350 (biphasic)	8
A. nidulans	WC (system I)	–	150	800 (biphasic)	19
Spinach	Cpl (system I)	–	150	800 (biphasic)	19
Spinach	RC 160 (system I)	–	150	800 (biphasic)	19
Spinach	TSF 1 (system I)	–	150	800 (biphasic)	19
Spinach	D 144 (system I)	–	150	800 (biphasic)	19
C. caldarium	WC (system I)	–	150	800 (biphasic)	19
Spinach	Cpl (system II)	–	150–170	∞	20
Secondary Units					
Chromatium D	WC	–	135	2.5	4
Chromatium D	WC	–	150	2.3	3
Chromatium D	WC	–		2	21
Chromatium viol.	WC	–		0.5–0.9	21
T. floridana	WC	–		0.4–0.8	21
R. palustris	WC	–		8–12	21
R. gelatinosa	WC	–		0.01–0.02	21
Rhodosporomonas sp *NW*	WC	–		0.002–0.006	21

Cph, chromatophores; WC, whole cells; Cpl, chloroplasts; RC, reaction centers; AUT-e, photoreceptor subunits (ref. 5); TSF 1, purified system I particle (ref. 20); D 144, purified system I particle (ref. 20).

can be answered by comparing T_{inf} observed in different systems. Table II lists these values and shows a rather surprising result; T_{inf} is virtually constant (ca. 150 K) for all but one of the systems listed. In other words, T_{inf}, which reflects the discontinuity in α, is the same in reaction centers (12, 17), phototrap complexes (5), chromatophores (5, 12, 15, 16), chloroplasts (18,19), bacterial and algae whole cells (18, 19) as well as purified particles of photosystems I and II (18, 19) and is the same in both primary and secondary (3, 4, 21) electron transport reactions.

The constancy of T_{inf} means that the source of the discontinuity in α is neither an energy property of each individual electron transport reaction nor a structural property of the lipids or water in the environment since the same T_{inf} is observed in membrane-free reaction centers and phototrap complexes as well as in dried films. Therefore, the source of the discontinuity is most likely the proteins or more correctly, a unique intramolecular bonding that occurs in proteins at ca. 150 K. Apparently, proteins in this more rigid configuration below 150 K possess a much smaller coefficient of thermal expansion, more typical of structured solids (see Table I), than at higher temperatures thereby giving rise to the near temperature independency of electron transport observed in region 2. For example, α for transport 1 is 4.2×10^{-5} K^{-1} in region 2 compared with 5.2×10^{-4} K^{-1} in region 1.

Unfortunately, to date little research has been undertaken to investigate the internal bonding and motional properties of proteins as a function of temperature. Two reasons for this obvious void of research are the lack of a probe that can be used to monitor information concerning the movement of proteins at low temperatures as well as the lack of interest in the physical properties of proteins at temperatures well below those of physiological importance.

There are, however, a few studies that have been concerned with the internal motions of proteins at these low temperatures. Hiltner et al. (25) measured the dynamic mechanical relaxation as a function of temperature from 4.2–500 K of several polyamino acids. Their data clearly show three relaxation maxima. Two of these occur at temperatures above room temperature and are, therefore, unimportant to this discussion. The third, on the other hand, occurs in the temperature region 105–135 K and has been interpreted by them as the freezing-in of side chair motions. It is, of course, impossible to state at this point whether or not there is a direct relation between the mechanism which stops protein side chain motion and the change in the coefficient of thermal expansion. Because of this, it would be of interest to determine the relaxation temperatures of naturally occurring proteins as opposed to the small polyamino acids used by Hiltner et al. (25) in order to see if the third relaxation temperature coincides with T_{inf} observed in electron transport studies.

Of more significance to this paper is the research recently published by Blumenfeld et al. (26) on the ability of adrenal ferredoxin to undergo conformational changes at low temperatures. Their study shows that redox-induced conformational rearrangements can only occur at temperatures above 150 K. It was further noted by them that this critical temperature is raised to ca. 190 K for ferredoxin in a water-ethylene glycol 50/50 mixture. This is consistent with the results of Clayton and Yau (17) who found

a T_{inf} of 190 K for the back primary photochemical reaction in bacterial reaction centers in a medium of 85% glycerol. As was mentioned above, this is the only electron transport reaction listed in Table I with a T_{inf} greatly different than 150 K. The reason for this difference in T_{inf} is not known. Apparently, however, in this system solvent molecules have some influence on the overall extent to which these proteins can contract.

Therefore, the temperature dependency that has been observed for the rate of various electron transport reactions can be explained in terms of the motion of the proteins to which the redox functional groups are bound. In such a model, electron transport occurs by electrons tunneling from one redox group to another, the rate of which is regulated mainly by the distance between the two groups. Implicit in this model is also the caution of investigating electron transport reactions at low temperatures. Since protein contraction can cause both an increase and decrease of the distance separating various redox groups, a temperature decrease can cause an electron transport reaction to occur at low temperatures that is nonexistent at physiological temperatures.

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